Environment supersedes host lineage in shaping the gut microbiome of squirrels in the Pacific Northwest

Undergraduate Research Thesis

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Abstract

Both host genetics and environmental factors have been shown to influence the establishment and composition of the gut microbiome, but it remains unclear whether host genetics or environment has a stronger effect in shaping the gut microbiome. The present study exploits a natural reciprocal transplant system of island and mainland *Tamiasciurus hudsonicus* (red squirrels) and *T. douglasii* (Douglas squirrels) in the Pacific Northwest to investigate the relative strength of genetic and environmental factors in influencing gut microbiome composition. Phylogeographic analysis of *Tamiasciurus* squirrels have shown a closer genetic relatedness between Vancouver Island (VI) red squirrels and interior mainland red squirrels despite the closer geographic proximity between VI red squirrels and coastal mainland Douglas squirrels. We extracted DNA from cecal samples of 35 red squirrels (13 VI and 22 mainland) and 34 Douglas squirrels specimens collected between 2008 and 2010 and amplified the V4 region of the bacterial 16s rRNA gene. The data were grouped by collection site, species identity, and geographic region, which we established based on the World Wildlife Fund's terrestrial ecoregion classifications that consider flora and fauna communities and environmental conditions. Surprisingly, our principal coordinate analysis (PCoA) plots for Bray-Curtis dissimilarity and weighted Uni-Frac distance showed VI red squirrels clustered with coastal mainland Douglas squirrels instead of with interior mainland red squirrels. Similarly, permutational analysis of variance (PERMANOVA) tests for Bray-Curtis and weighted Uni-Frac showed a large distance between the VI and interior mainland red squirrels. Altogether, these results suggest that the environment is a stronger driver of the gut microbiome in *Tamiasciurus* in the Pacific Northwest than host lineage is. Our findings from this natural reciprocal transplant system provide additional support to a growing number of studies indicating that the

environment overshadows phylogenetic effects of the host species in shaping the gut microbiome.

Introduction

The gut microbiome is a dynamic matrix of microorganisms in the gastrointestinal tract (Thursby & Juge, 2017), and in recent years, numerous studies have connected the gut microbiome with roles in health status, immunity, and digestion (Kho & Lal, 2018; Round & Mazmanian, 2009; Hooper et al., 2002). This microbial environment consists of both commensal and pathogenic microbiota, including bacteria, fungi, viruses, and protozoa (Thursby & Juge, 2017). Compositionally, the gut microbiome varies along the gastrointestinal tract, with obligate anaerobes occupying the lower gastrointestinal tract in a higher proportion than in the upper tract (Suzuki & Nachman, 2016).

However, the gut microbiome has also been shown to transform in response to other factors as well, such as host genetics and the environment. The host and its gut microbiome appear to have a phylosymbiotic relationship, in which the relatedness of host-associated microbes recapitulates the phylogenetic interactions between related host species (Brooks et al., 2016). Variations in host genetics alter the phenotype of the host, which in turn affects the composition of gut microbiota and their phenotypes as well (Goodrich et al., 2014). In addition, human twin studies show greater similarity in gut microbiome compositions between related individuals compared with unrelated individuals, suggesting heritability of microbial taxonomic groups (Lee et al., 2011). However other studies reveal that these familial similarities in gut microbiome may be an effect of shared environment or dietary preferences (David et al., 2013). Although dietary preferences can be viewed as a phylogenetic effect (Knowles et al., 2019), the natural availability of food resources for wild animals is heavily influenced by the environment.

Numerous studies have found that diet and other environmental factors—such as forest type, co-housing, and season—influence the gut microbiome and may even be a stronger driver of its composition than host genetics is (Carmody et al, 2015; Amato et al., 2015; Seedorf et al., 2014; Maurice et al., 2015).

Although numerous studies show that both host genetics and the environment influence the composition of the gut microbiome, it is currently unclear which one has a stronger influence in shaping the microbiome. The present study investigates the relative strengths of both factors by examining a natural reciprocal transplant experiment involving congeneric red squirrels (*Tamiasciurus hudsonicus*) and Douglas squirrels (*Tamiasciurus douglasii*) in the Pacific Northwest.

Red squirrels and Douglas squirrels diverged over 400,000 years ago in the middle Pleistocene era, and more recently, the lineage of red squirrels split between interior mainland populations in the Pacific Northwest and the populations inhabiting Vancouver Island (Chavez et al., 2013). Despite being more closely related to the interior mainland population of red squirrels, Vancouver Island red squirrels are geographically proximate to coastal Douglas squirrels. Unlike interior mainland red squirrels, which feed primarily on lodgepole pinecones and occupy a relatively dry and arid habitat, Vancouver Island red squirrels inhabit a coastal environment—similar to that of coastal Douglas squirrels—which fosters forests of Douglas-fir (Chavez et al., 2011).

Based on the dynamics of this natural reciprocal transplant system, we formulated two predictions (Figure 1). 1) If the environment has a stronger effect on the gut microbiome composition, then we expect the gut microbiomes of Vancouver Island red squirrels to resemble those of coastal Douglas squirrels. 2) If host lineage has a stronger effect on the gut microbiome

composition, then we expect the gut microbiomes of Vancouver Island red squirrels to resemble those of interior mainland red squirrels.

Methods

Sample Collection

Squirrel specimens were collected between 2008 and 2010 *via* the use of firearms or by opportune recovery of roadkill. In total, 35 red squirrel specimens—13 from Vancouver Island and 22 from Washington state (interior mainland)—and 34 Douglas squirrel specimens were collected from various sites in Washington state, British Columbia, and Oregon (Figure 2). One additional specimen was excluded from analyses due to potentially having an unnatural diet during its time at a wildlife rehabilitation center. Geographic coordinates of each specimen were recorded and then associated with ecoregions based on the World Wildlife Fund's Terrestrial Ecoregions of the World (Table 1).

DNA Extraction and Amplification

Microbial DNA from cecal samples was extracted using the QIAamp DNA Stool Mini Kit (Qiagen) in accordance with the protocol developed by Smith et al. (2011). 0.2g of sterile zirconia/silica beads were added to the sample, and the mixture was vortexed for homogenization and mechanically disrupted with TissueLyser LT (Qiagen) at 30HZ for 6 minutes. The subsequent suspension was heated at 95°C for 5 minutes, and microbial DNA was extracted after vortexing and centrifuging the sample. To check the quality of the extracted DNA, we used NanoDrop 3300 (Thermo Fisher Scientific).

We then amplified the bacterial V4 region of the 16s rRNA in accordance with the protocol developed by Caporaso et al. (2010). The genetic material was sequenced on a MiSeq PE 251X251 + 12 bp index from University of California, Davis with primer sequences 515F

(Parada)–806R (Apprill), forward-barcoded: FWD:5' GTGYCAGCMGCCGCGGTAA; REV:5' GGACTACNVGGGTWTCTAAT.

QIIME2 Analysis

We used QIIME2 (Bolyen et al., 2019), a microbiome bioinformatics software, for our analyses. We ran pair-end sequencing and then demultiplexed and used the default parameters for quality filtering. The data were denoised with the DADA2 denoise-paired function. The sequences were clustered into taxonomic groups using the Greengenes 13_8 reference database (DeSantis et al., 2006) with a minimum sequencing identity of 99%. We used the metrics-phylogeny function to calculate alpha-diversity metrics per individual and measure the differences in proportion of operational taxonomic units (OTU's) per individual (beta diversity).

Taxonomic Bar Plots

After analyzing the data with QIIME2, we visualized the relative abundances of microbial taxonomic group present in each host as a stacked bar plot using the *tidyverse* package in R (Wickham et al., 2019; R Core Team, 2020). We created taxonomic bar plots at the phylum and genus levels.

Diversity Metrics

Both alpha- and beta- diversity metrics were computed using QIIME2, and all pairwise comparisons were corrected for multiple tests using Benjamini-Hochberg False Discovery Rate (FDR) correction, resulting in the q-value statistic (Benjamini & Hochlberg, 1995).

Alpha-diversity metrics analyzed in the present study include species richness (Shannon index), species evenness (Pielou's evenness index), and phylogenetic distance (Faith's phylogenetic distance). Species richness considers the number of unique species represented an individual's gut microbiome community. Species evenness describes the relative abundance of

the different microorganism species in the microbiome and places more weight on the spread of individuals across species in the microbiome than the richness metric does. Phylogenetic distance calculates the genetic relatedness between microorganisms in the microbiome and estimates the time that each individual microorganism has evolved from each other.

Beta-diversity metrics analyzed in the present study include non-phylogenetic metrics (Bray-Curtis dissimilarity) and phylogenetic metrics (weighted and un-weighted Uni-Frac distances). Bray-Curtis dissimilarity quantifies the dissimilarity in composition between the microbiomes of different hosts. Like non-phylogenetic metrics, Uni-Frac distances compare the differences in microbial communities between hosts, but Uni-Frac distances also consider the genetic relatedness and phylogenetic distances between microorganisms within the gut microbiome. Unweighted Uni-Frac distances is based solely on the presence or absence of taxonomic groups and does not consider relative abundances, whereas weighted Uni-Frac distances considers the abundances of each taxonomic group. We visualized these metrics with principal coordinate analysis (PCoA) plots and ran permutational analysis of variance (PERMANOVA) and homogeneity of multivariate dispersion (PERMDISP) statistical analysis tests on the beta diversity metrics using QIIME2.

Results

Relative Abundances of Taxonomic Groups

In both red squirrels and Douglas squirrels, the bacterial phyla *Firmicutes* and *Bacteroidetes* appeared to have the greatest relative abundances (Figure 3). Although *Firmicutes* is the dominant phylum for both red and Douglas squirrels, interior mainland red squirrels overall had a greater abundance (mean = 0.42) and consistency of *Bacteroidetes* than both Vancouver Island red squirrels (mean = 0.25) and coastal Douglas squirrels (mean = 0.20), with

32% of interior mainland red squirrels having a relative abundance of *Bacteroidetes* greater than 0.50. At the genus level (Figure 4), *Prevotella* from the *Bacteroidetes* phylum appears to have a consistently high relative abundance in interior mainland red squirrels (mean = 0.22), especially when compared with Vancouver Island red squirrels (mean = 0.069) and coastal Douglas squirrels (0.077).

Alpha-diversity Metrics

After running pairwise Kruskal-Wallis one-way analysis of variance tests on Shannon index, Pielou's evenness index, and Faith's phylogenetic diversity, all samples showed no significant difference (q-value > 0.05) between Vancouver Island red squirrels, interior mainland red squirrels, and coastal Douglas squirrels. In addition, when samples were grouped by sex, collection season, collection month, and ecoregion, there were no significant differences in all three alpha-diversity metrics.

Beta-diversity Metrics

PCoA plots for Bray-Curtis dissimilarity and weighted and unweighted Uni-Frac distance showed that interior mainland red squirrels formed a relatively isolated cluster, whereas Vancouver Island red squirrels overlap and cluster with coastal Douglas squirrels and not interior mainland red squirrels (Figure 5). At a finer classification level based on collection site, Vancouver Island red squirrels overlap most with coastal Douglas squirrels collected along the transect in Washington state. Axis 1 explained most of the variance in all plots (Bray-Curtis = 10.52%; weighted Uni-Frac = 9.684%; unweighted Uni-Frac = 42.95%).

As shown in Table 2, all PERMANOVA tests were computed with 999 permutations, resulting in the q-value statistic. Results from PERMANOVA tests on the Bray-Curtis dissimilarity metric indicate that the comparison between interior mainland red squirrels and

Vancouver Island red squirrels has a statistically significant (q-value < 0.05) and relatively large pseudo-F (5.824447), which is a statistical value that measures the differences between groups. The pseudo-F values when comparing interior mainland red squirrels with subgroups of coastal Douglas squirrels—British Columbia (3.986067), Oregon (4.426762), and Washington (4.735420)—were statistically significant (q-values < 0.05). Comparisons between Vancouver Island red squirrels and all subgroups of coastal Douglas squirrels were also statistically significant, but the pseudo-F values were smaller, ranging from 2.047867 to 2.987009. Similarly, PERMANOVA tests for unweighted Uni-Frac distances yielded parallel results when comparing interior mainland red squirrels and Vancouver Island red squirrels (pseudo-F = 5.414587, q-value < 0.05), interior mainland red squirrels and subgroups of coastal Douglas squirrels (pseudo-F values between 3.547723 and 4.109804, q-values < 0.05), and Vancouver Island red squirrels and subgroups of coastal Douglas squirrels (pseudo-F values between 2.013941 and 3.038627, q-values < 0.05). PERMANOVA results for weighted Uni-Frac distance showed statistically significant (q-value < 0.05) comparisons between interior mainland red squirrels and Vancouver Island red squirrels (pseudo-F = 7.311387) as well as interior mainland red squirrels and subgroups of coastal Douglas squirrels (pseudo-F values between 7.283111 and 7.467468). However, comparisons between Vancouver Island red squirrels and subgroups of coastal Douglas squirrels were not statistically significant, with pseudo-F values ranging from 1.265638 to 2.594804.

Results from PERMDISP tests for each group comparison in all three beta-diversity metrics were statistically insignificant (q-value > 0.05).

Discussions

We found that the environment plays a stronger role in shaping the gut microbiome than host lineage is. The independent clustering of interior mainland red squirrels on the PCoA plots for all beta-diversity metrics shows that the gut microbiome compositions of interior mainland red squirrel individuals are more similar to each other than they are to the compositions of any other group. On the other hand, Vancouver Island red squirrels clustered with coastal Douglas squirrels, especially with those collected in Washington, indicating that the gut microbiome compositions of Vancouver Island red squirrels more closely resemble those of coastal Douglas squirrels than they do to interior mainland red squirrels. Interestingly, despite being in the same terrestrial ecoregion classification, Vancouver Island red squirrel and Oregon Douglas squirrel clusters did not overlap in the Bray-Curtis and weighted Uni-Frac PCoA plots. This could potentially be a result of climatic variations or differences in human interactions that exist on this longitudinal gradient, as the distance between the Oregon collection sites and the rest reach up to 700 kilometers.

Other analyses also support our PCoA interpretations. PERMANOVA tests on all beta-diversity metrics suggest that environment supersedes host lineage in shaping the gut microbiome s. PERMANOVA of Bray-Curtis dissimilarity shows that Vancouver Island red squirrels and interior mainland red squirrels had the greatest computed difference in variation of microbial abundance, as indicated by a pseudo-F value of 5.824447 (q-value < 0.05), followed by comparisons between interior mainland red squirrels and all subgroups of coastal Douglas squirrels (pseudo-F values between 3.986067 and 4.735420, q-values < 0.05). These relatively large and significant pseudo-F values support the idea that the gut microbiome compositions of interior mainland red squirrels are significantly different from those of Vancouver Island red squirrels and coastal Douglas squirrels. Furthermore, pairwise Bray-Curtis PERMANOVA

comparisons between Vancouver Island red squirrels and all subgroups of coastal Douglas squirrels resulted in smaller, statistically significant pseudo-F values than the comparison between interior mainland red squirrels and Vancouver Island red squirrels, suggesting that the compositional abundance of microbes in the gut of Vancouver Island red squirrels are more similar to those of all coastal Douglas subgroups than they are to those of interior mainland red squirrels. Like with our interpretation of the PCoA plots, the Bray-Curtis PERMANOVA results suggest a smaller variance between Vancouver Island red squirrels and Washington Douglas squirrels, but no additional tests were computed to determine the statistical significance of this observation.

The PERMANOVA results for unweighted Uni-Frac distances were similar to those for Bray-Curtis dissimilarity. Unweighted Uni-Frac distances consider phylogenetic relatedness between gut microorganisms based on presence or absence without factoring in the relative abundances of these microbes. Thus, these results indicate that the gut microorganisms found in Vancouver Island red squirrels are more related to those found in coastal Douglas squirrels than they are to those found in interior mainland red squirrels. However, when considering abundance with weighted Uni-Frac distances, only comparisons between interior mainland red squirrels and all other groups showed significant results, indicating that the gut microbiomes of interior mainland red squirrels are not made up of similar taxa with similar abundances as those of Vancouver Island red squirrels and coastal Douglas squirrels. The comparison between Vancouver Island red squirrels and all subgroups of coastal Douglas squirrels yielded relatively small pseudo-F values but without statistical significance. This means we cannot reject the null hypothesis, which states that Vancouver Island red squirrels and the subgroups of coastal Douglas squirrels are equivalent or show no difference in weighted Uni-Frac distance metrics. In

other words, there is a chance that the gut microbiomes of Vancouver Island red squirrels closely resemble those of coastal Douglas squirrels in terms of related taxa and abundances. Furthermore, our PERMDISP results for all three beta diversity metrics were insignificant (q-values > 0.05), which confirms that the variations we observed with our PERMANOVA tests are a result of differences in the average community composition and not an artifact of heterogenous dispersion.

The two squirrel species differed from each other in their dominant gut bacterium, as visualized with the taxonomic bar plots. Vancouver Island and coastal Douglas squirrels are dominated by *Firmicutes*, whereas interior mainland red squirrels had nearly equivalent proportions of *Bacteroidetes* and *Firmicutes* phyla. *Bacteroidetes* is found in the environment and as a part of the normal gut microbiome where it acts as mutualistic symbiont capable of degrading large, complex biopolymers in the large intestine (Thomas et al., 2011). Within *Bacteroidetes*, the genus *Prevotella* appears to be the consistently dominant genus in interior mainland red squirrels while Vancouver Island red squirrels and coastal Douglas squirrels have no clear dominant genus. The impact of host lineage or environment on specific taxa and the potential for certain genera like *Prevotella* to act as indicators of host species or environmental conditions remains to be explored.

Conclusion

Congeneric red and Douglas squirrels in the Pacific Northwest form a unique system that resembles a natural reciprocal transplant experiment, in which red squirrels on Vancouver Island display a closer genetic relatedness to interior mainland red squirrels, but these Vancouver Island populations geographically proximate to coastal Douglas squirrels. The relative strengths of the environment and host lineage on the gut microbiome determines whether the microbial

compositions of Vancouver Island red squirrels will resemble those of coastal Douglas squirrels, which inhabit a similar environment, or interior mainland red squirrels, which are more closely related.

From our analyses, the gut microbiome compositions of Vancouver Island red squirrels were significantly different from those of interior mainland red squirrels and instead resembled those of coastal Douglas squirrels. This suggests that the environment acts as a stronger driver of the gut microbiome in *Tamiasciurus* in the Pacific Northwest than host lineage is. Our findings from this natural reciprocal transplant system provide additional support to a growing number of studies indicating that the environment overshadows phylogenetic effects of the host species in shaping the gut microbiome.

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Figures & Tables

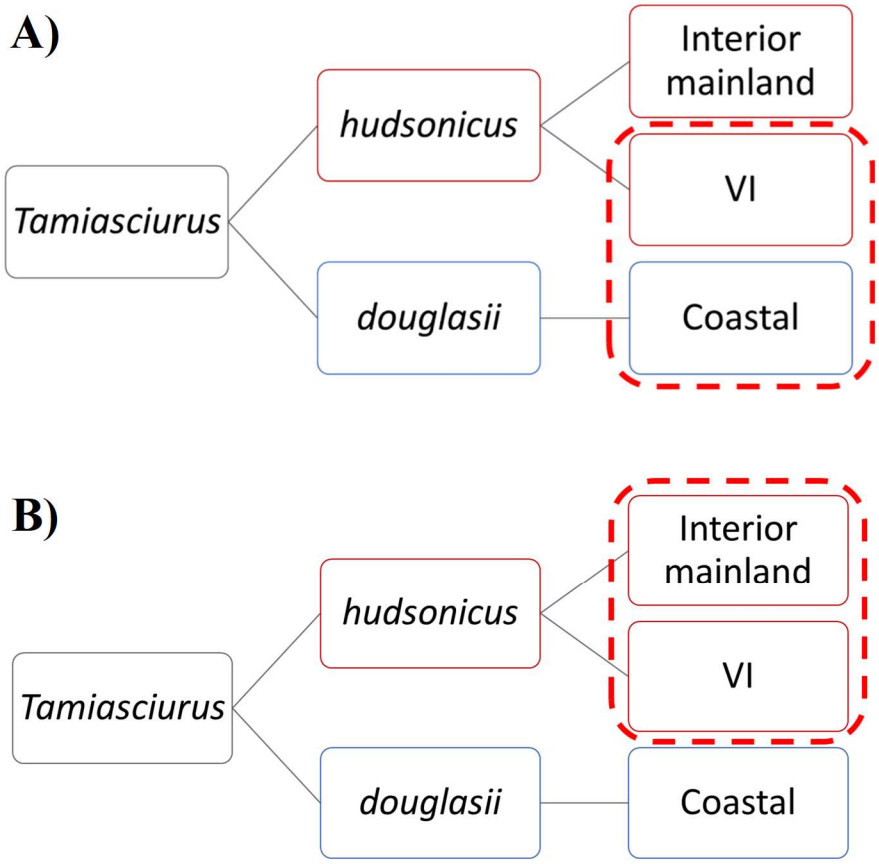

Figure 1. A) Prediction 1: if the environment has a stronger effect on the gut microbiome, then the gut microbiome compositions of VI TH will resemble those of coastal TD. B) Prediction 2: if host lineage has a stronger effect on the gut microbiome, then the gut microbiome compositions of VI TH will resemble those of interior mainland TH.

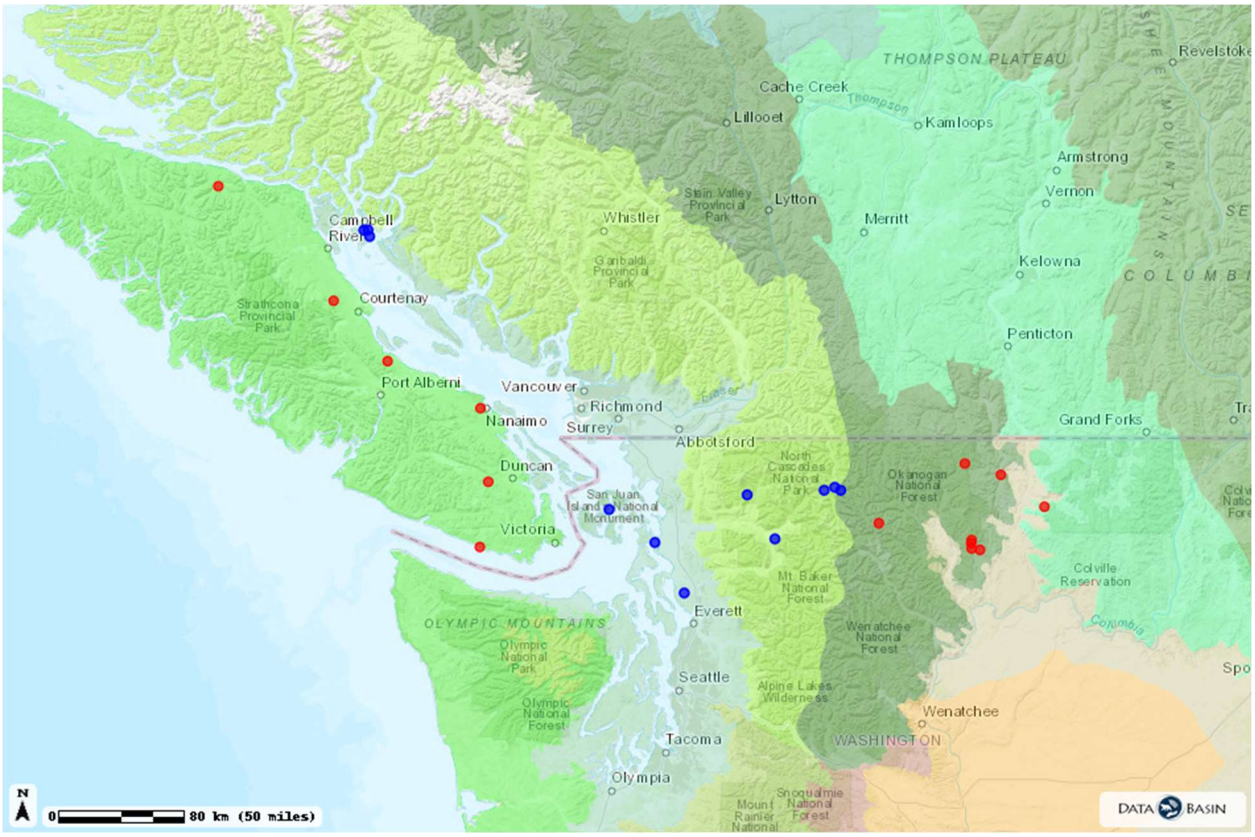

Figure 2. Distribution of *Tamiasciurus* collection sites in Vancouver Island (left) through interior mainland Washington (right). Red points correspond with red squirrels, and blue points, with Douglas squirrels. Oregon TD collection sites are not shown.

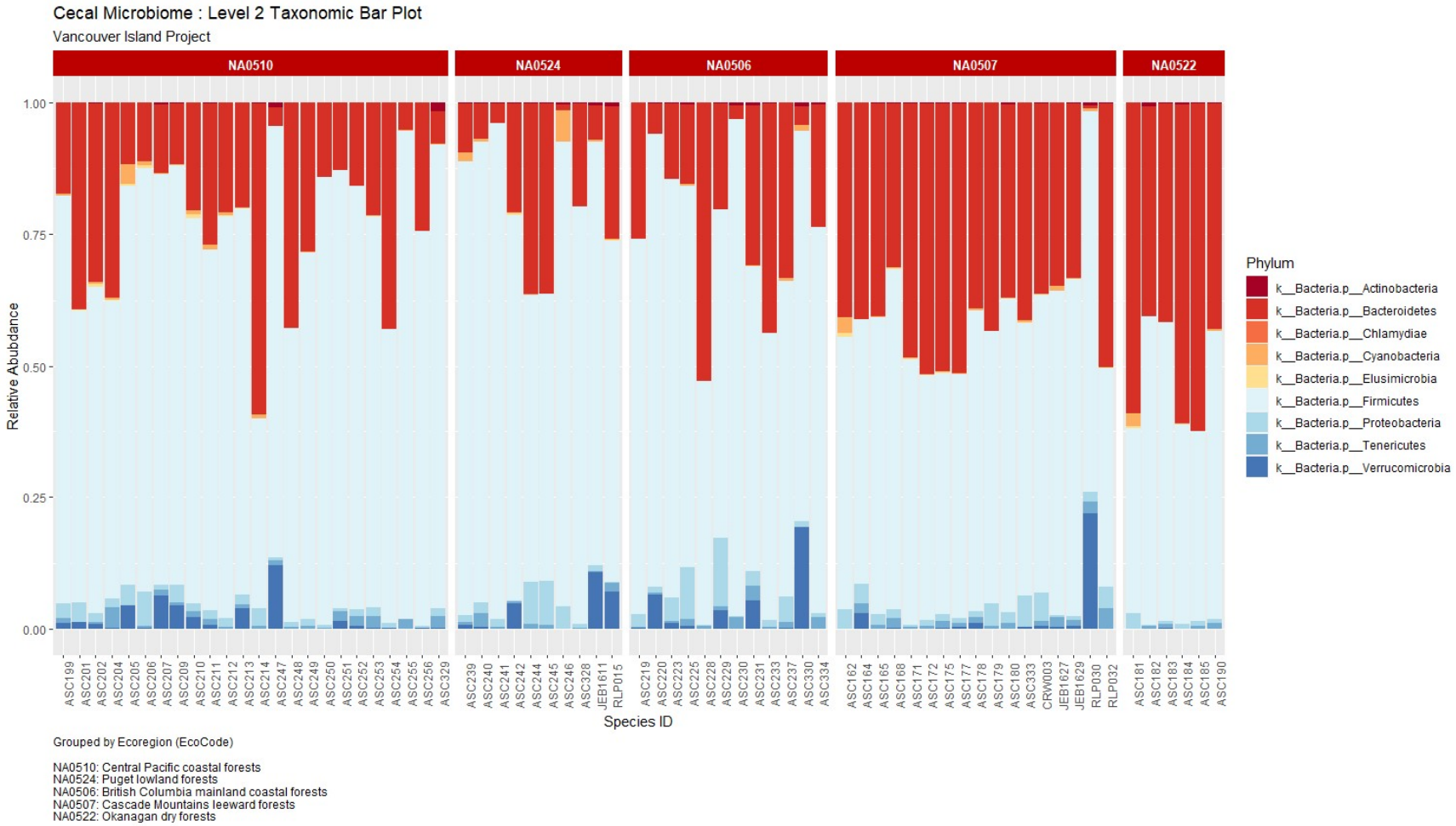

Figure 3. Taxonomic bar plot at the phylum level. From left to right: ecoregion NA0510 = VI TH and coastal TD (Oregon), NA0524 = coastal TD (Washington and British Columbia), NA0506 = coastal TD (Washington), NA0507 = interior mainland TH (Washington), NA0522 = interior mainland TH (Washington).

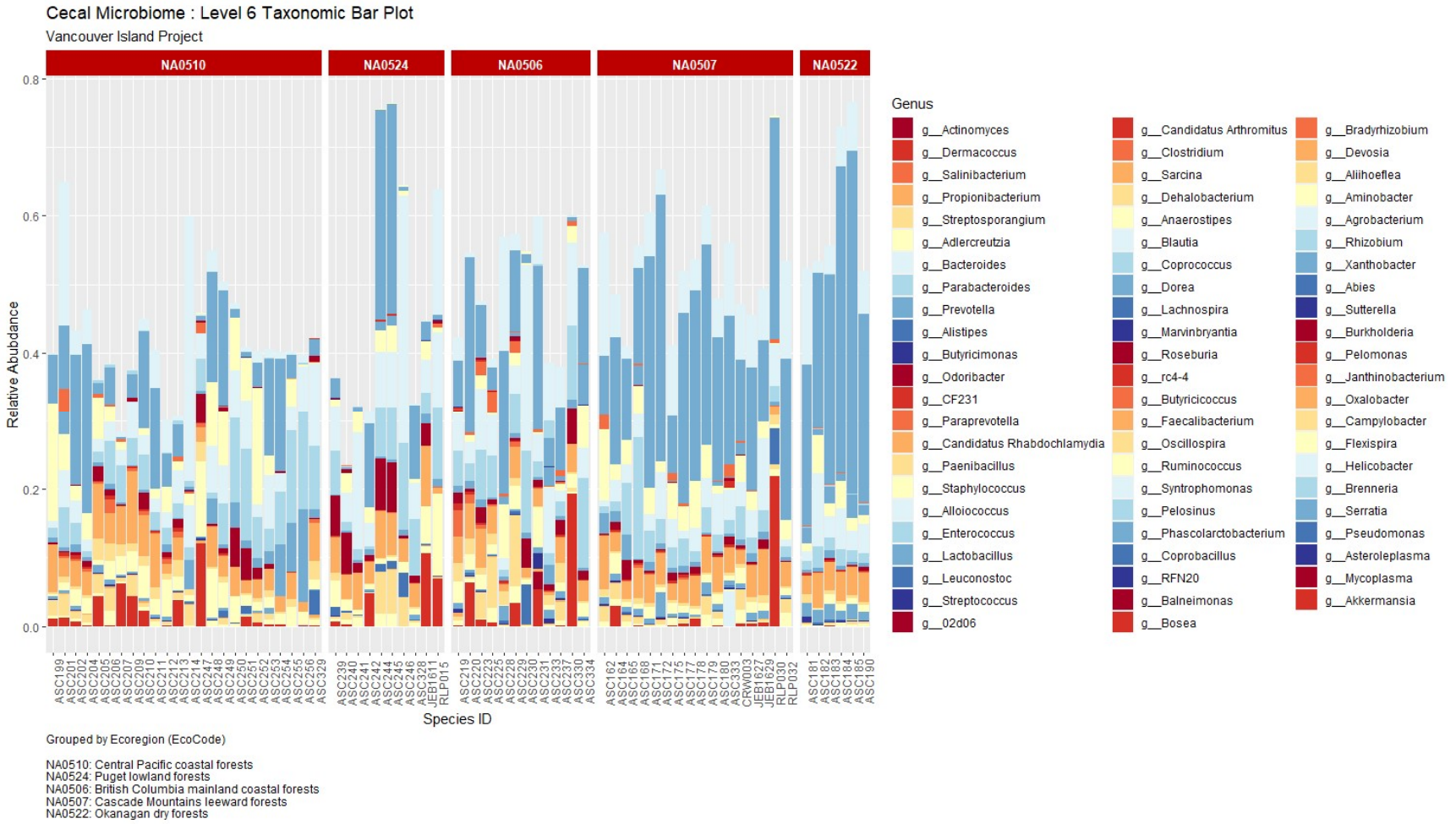

Figure 4. Taxonomic bar plot at the genus level. From left to right: ecoregion NA0510 = VI TH and coastal TD (Oregon), NA0524 = coastal TD (Washington and British Columbia), NA0506 = coastal TD (Washington), NA0507 = interior mainland TH (Washington), NA0522 = interior mainland TH (Washington). Empty bars are due to lack of data refinement at the genus level.

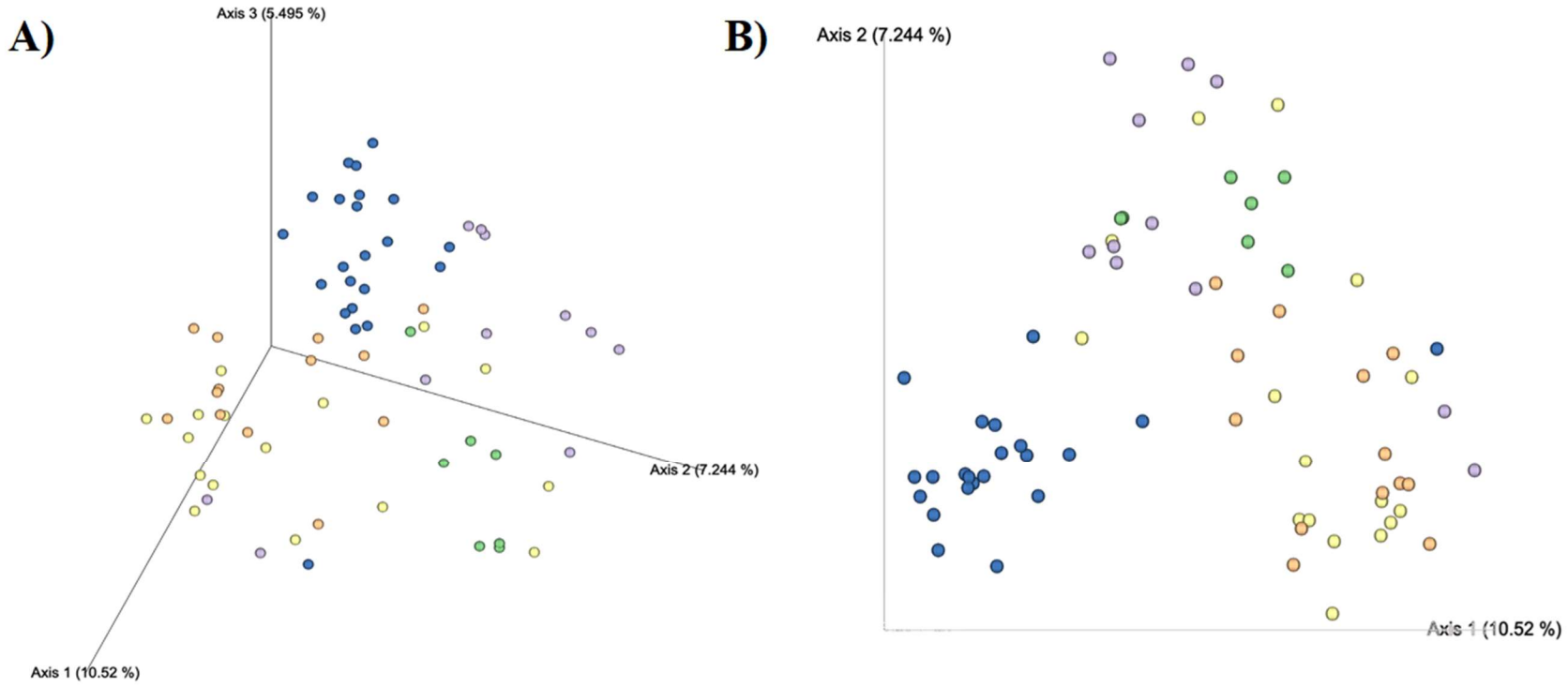

Figure 5. Principal coordinate analysis plots of A) weighted Uni-Frac distance and B) Bray-Curtis dissimilarity. Blue = interior mainland TH, orange = VI TH, yellow = coastal TD (Washington), purple = coastal TD (Oregon), green = coastal TD (British Columbia). Axis 1 explains most of the variance in both plots (weighted Uni-Frac = 9.68%; Bray-Curtis = 10.52%).

Code	Ecoregion	Collection Site	Description Summaries
NA0510	Central Pacific coastal forests	VI red; coastal Douglas (Oregon)	- Mean annual temperature: 8.5-13°C - Rainfall: 1500-3500 mm/year - Climate: warm summers, mild winters - Major forests: Douglas-fir and western hemlock - Characteristic wildlife: migratory shore birds, sea birds, elk, black-tailed deer, coyote, black bear, mink, raccoon
NA0524	Puget lowland forests	coastal Douglas (British Columbia; Washington)	- Mean annual temperature: 9°C - Rainfall: 800-900 mm/year - Climate: "Mediterranean-like"; warm/dry summers, mild/wet winters - Major forests: western red cedar, western hemlock, and Douglas-fir - Characteristic wildlife: raccoon, sea otter, mink, coyote, black-tailed deer, harbor seal, turkey vulture, bald eagle, migratory shorebirds, seabirds
NA0506	British Columbia mainland coastal forests	coastal Douglas (Washington)	- Mean annual temperature: 5-6.5°C - Rainfall: 1200-4500 mm/year - Climate: gradient, with slightly drier/cooler interior - Major forests: western hemlock, western red cedar, amabilis fir - Characteristic wildlife: black-tailed deer, black and grizzly bears, mountain goat, wolf, mink, Northern river otter, waterfowl, moose, red fox, marten
NA0507	Cascade Mountains leeward forests	interior mainland red	- Mean annual temperature: 3.5-6°C - Rainfall: 300-1200 mm/year - Climate: gradient of moist coastal to semi-arid continental - Major forests: Engelmann spruce, subalpine fir, and lodgepole pine - Characteristic wildlife: bighorn sheep, mountain goat, grizzly and black bears, coyote, blue grouse, cougar, raptors, salmon, spotted owl
NA0522	Okanagan dry forests	interior mainland red	- Mean annual temperature: 7°C - Rainfall: 250-1000 mm/year - Climate: very warm-hot/dry summers, moderately cool winters - Major forests: lodgepole pine, quaking aspen, white spruce, and Douglas-fir - Characteristic wildlife: black bear, Northern river otter, bighorn sheep, black- and white-tailed deer, American badger, waterfowl, long-billed curlew

Table 1. Classification of samples based on Terrestrial Ecoregions of the World (Olson et al., 2001) and descriptions of ecoregions.

				Bray-Curtis		
Group 1	**Group 2**	**Sample Size**	**Permutations**	**pseudo-F**	**p-value**	**q-value**
CI_TD	OR_TD	18	999	2.047867	0.001	0.001429
	VI_TH	20	999	2.745026	0.001	0.001429
	WA_TD	23	999	2.195918	0.002	0.002000
	WA_TH	29	999	3.986067	0.001	0.001429
OR_TD	VI_TH	24	999	2.987009	0.002	0.002000
	WA_TD	27	999	2.345761	0.002	0.002000
	WA_TH	33	999	4.426762	0.001	0.001429
VI_TH	WA_TD	29	999	2.433470	0.001	0.001429
	WA_TH	35	999	5.824447	0.001	0.001429
WA_TD	WA_TH	38	999	4.735420	0.001	0.001429
				Unweighted Uni-Frac		
Group 1	**Group 2**	**Sample Size**	**Permutations**	**pseudo-F**	**p-value**	**q-value**
CI_TD	OR_TD	18	999	2.013941	0.004	0.004000
	VI_TH	20	999	3.038627	0.001	0.001250
	WA_TD	23	999	2.097677	0.003	0.003333
	WA_TH	29	999	3.971718	0.001	0.001250
OR_TD	VI_TH	24	999	2.781578	0.001	0.001250
	WA_TD	27	999	2.422772	0.001	0.001250
	WA_TH	33	999	4.109804	0.001	0.001250
VI_TH	WA_TD	29	999	2.547734	0.001	0.001250
	WA_TH	35	999	5.414587	0.001	0.001250
WA_TD	WA_TH	38	999	3.547723	0.001	0.001250
				Weighted Uni-Frac		
Group 1	**Group 2**	**Sample Size**	**Permutations**	**pseudo-F**	**p-value**	**q-value**
CI_TD	OR_TD	18	999	1.593660	0.119	0.146667^
	VI_TH	20	999	2.594804	0.035	0.070000^
	WA_TD	23	999	1.693550	0.132	0.146667^
	WA_TH	29	999	7.283111	0.001	0.002500
OR_TD	VI_TH	24	999	1.937954	0.063	0.105000^
	WA_TD	27	999	1.265638	0.234	0.234000^
	WA_TH	33	999	7.321376	0.001	0.002500
VI_TH	WA_TD	29	999	1.480251	0.132	0.146667^
	WA_TH	35	999	7.311387	0.001	0.002500
WA_TD	WA_TH	38	999	7.467468	0.001	0.002500

Table 2. PERMANOVA test results for beta-diversity metrics. All pairwise comparisons were corrected for multiple tests using Benjamini-Hochberg FDR correction (q-value) (Benjamini & Hochberg, 1995). All q-values are significant (q-value < 0.05) except those marked by ^. CI_TD = coastal TD (Cortes Island, British Columbia), OR_TD = coastal TD (Oregon), VI_TH = VI TH, WA_TD = coastal TD (Washington), WA_TH = interior mainland TH.